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Trend Analysis of Antimicrobial Resistance in *Campylobacter jejuni* and *Campylobacter coli* Isolated from Belgian Pork and Poultry Meat Products Using Surveillance Data of 2004–2009

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Abstract

The purpose of this study was to analyze and compare antimicrobial resistance in *Campylobacter* spp. isolated from pork and poultry carcasses, and pork and poultry meat (at slaughterhouse level, during meat cutting, and at retail) in Belgium, using available surveillance data over the period 2004–2009. The susceptibilities of 1724 *Campylobacter* isolates for ampicillin, ciprofloxacin, nalidixic acid, tetracycline, erythromycin, and gentamicin were tested by E-test. Gentamicin resistance was low (near 0%) until 2007, with an increase to over 20% by 2009 for all species-matrix combinations. Resistance to tetracycline fluctuated around the same level during the entire study period and was significantly higher (p-value of < 0.05) in *C. coli* than in *C. jejuni*. Erythromycin resistance was low and showed a slight decrease between 2004 and 2007, but increased from 2007 until 2009. Fluoroquinolone and ampicillin resistance was significantly higher in isolates derived from poultry, compared to pork-related isolates. This correlates with the higher use of these antimicrobials in poultry husbandry. A total of 25% of *C. coli* isolates from poultry showed the most apparent multiresistance (resistance to four or more antimicrobials). Approximately 1% of the poultry-derived isolates (both *C. coli* and *C. jejuni*) showed resistance to all tested antimicrobials, while none was found in pork products.

Introduction

Campylobacter is the most common cause of bacterial gastroenteritis worldwide, most frequently caused by *C. jejuni*, followed by *C. coli* and *C. lari* (ESFA, 2009). Since 2005, campylobacteriosis has been the most reported zoonotic infection in Belgium, with 5635 laboratory-confirmed cases in 2009 (53 per 100,000 inhabitants), although since 2000 the number of *Campylobacter* infections has shown a significant decreasing trend at the national and regional levels (Belgian Working Group, 2010). Although campylobacteriosis is often self-limiting, antibiotic treatment may be necessary in severe cases (Molbak, 2005). The treatment of choice had traditionally been macrolides or fluoroquinolones, for which *Campylobacter* spp. were historically susceptible. However, increased resistance to both groups of antimicrobials has been observed (Belanger and Shryock, 2007; Luangtongkum *et al.*, 2009). In addition, drug-resistant foodborne bacteria have a selective advantage in patients already treated with antimicrobial drugs for other reasons, resulting in increased transmission (Perron *et al.*, 2008). Also, the genes encoding for antimicrobial drug resistance are often located on mobile genetic elements, such as plasmids, transposons, and integrons, which can be horizontally transferred to other bacteria, possibly pathogens (Avrain *et al.*, 2004; Jeon *et al.*, 2008). It has been shown that drug-resistant bacteria show increased virulence, causing a longer duration of illness (Nelson *et al.*, 2004).

Due to the zoonotic nature of campylobacteriosis, the intensive use of antimicrobial agents for therapy, prophylaxis, or growth promoters in food animal production (although the latter has been prohibited in Europe since 2006) can contribute considerably to increased resistance in clinical *Campylobacter* isolates (EFSA, 2009, 2010; Aerestrup and Wegener, 1999). Several European countries have monitoring programs to assess and mitigate the risk related to antimicrobial resistance (Ammon and Makela, 2010). The main mode of transmission is the consumption of undercooked meat or contamination by the food handler of fresh poultry and pork meat (Corry and Atabay, 2001; Altekruse and Tollefson, 2003; Cools *et al.*, 2005). Since 2000, the contamination of pork and poultry carcasses and meat with *Campylobacter* spp. has been monitored in Belgium. The proportion of positive poultry samples

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Table 1. Percentage of *Campylobacter*-Positive Samples in Belgium

Matrix	*2004*	*2005*	*2006*	*2007*	*2008*	*2009*
Poultry carcass at slaughterhouse[a]	27.9 ($n=197$)	19.6 ($n=270$)	19.0 ($n=315$)	22.5 ($n=235$)	33.0 ($n=185$	32.0 ($n=261$)
Poultry meat at processing[a]	26.0 ($n=131$)	22.9 ($n=249$)	12.4 ($n=326$)	9.3 ($n=257$)	7.3 ($n=523$)	8.6 ($n=513$)
Poultry carcass at retail[b]	35.0 ($n=77$)	3.9 ($n=77$)	42.5 ($n=40$)	19.4 ($n=144$)	19.1 ($n=115$)	25.4 ($n=118$)
Pork carcass at slaughterhouse[c]	5.0 ($n=344$)	7.2 ($n=433$)	13.4 ($n=418$)	12.2 ($n=213$)	16.6 ($n=500$)	14.0 ($n=656$)

[a]Sample size of 1 g.
[b]Sample size of 0.01 g.
[c]Sample size of 600 cm^2.

shows strong annual fluctuations but overall remains high (Table 1) (Trends and Sources, 2004–2009). Since 2004, these strains have been tested for antimicrobial resistance. Belgium does not yet have a central detailed surveillance system in place on each level of antimicrobial consumption or antimicrobial resistance, in contrast to some other European countries such as Denmark and Sweden. These countries annually publish detailed reports on the general use of antimicrobials and occurrence of antimicrobial resistance in food animals, foods, and humans. Recent efforts have been made by the European Medicines Agency (EMA, 2009) to develop a central database on the use of antimicrobial agents in animals (i.e., European Surveillance of Veterinary Antimicrobial Consumption [ESVAC]). Belgium is also working on a program for surveillance of antimicrobial use in agriculture; however, linking and explaining different observed trends in antimicrobial use and resistance development remains difficult.

The aim of the present study is to analyze and compare antimicrobial resistance proportions in *Campylobacter* spp. isolated from food samples (i.e., pork and poultry carcasses and meat) from Belgium through surveillance data over the period 2004–2009. Six antimicrobials were tested, including those that are recommended for therapy. Focus was placed on detection of reduced susceptibility using European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiological cut-off values.

Methods

Sampling and isolation of Campylobacter *isolates*

Samples of pork and poultry carcasses, meat, and meat products were taken by inspectors of the Belgian Federal Agency for the Safety of the Food Chain (FASFC) as part of continuous surveillance plans, as laid down in Belgian or European Legislation. Sampling was done at random at the level of production (i.e., at the slaughterhouse directly after slaughter, during meat cutting, and at retail). Samples were kept at 4°C until analysis. Food stuffs sampled were pork carcasses, broilers, spent hens, turkey carcasses, poultry parts, poultry meat preparations, and minced poultry meat.

Campylobacter isolates from food were obtained as described by ISO 10272-1 and 10272-2 (ISO, 2006). After isolation, the strains were sent to the national reference laboratory of *Campylobacter* at the Institute of Public Health (Brussels, Belgium).

Identification of species

DNA was extracted through heat lysis (Englen and Kelley, 2000). One colony was suspended in 100 μL of MQ water, boiled for 17 min, and chilled immediately. Three different polymerase chain reaction (PCR) protocols were utilized to confirm all isolates as belonging to the *Campylobacter* genus (Denis *et al.*, 2001) and to identify the isolates as *C. jejuni* or *C. coli* by molecular weight (Debruyne *et al.*, 2008; Denis *et al.*, 1999; Linton *et al.*, 1997). Primers are listed in Table 2.

Minimal inhibitory concentration (MIC) determination

Since the E-test has been found to compare favorably with agar dilution methods (Luber *et al.*, 2003; Oncul *et al.*, 2003), this test was chosen for its ease of use in the routine testing of single isolates. Confirmed *Campylobacter* isolates were subcultured on Colombia agar with 5% sheep blood (Oxoid Ltd., Hampshire, UK) for 24 h at 42°C under micro-aerophilic conditions, using sealed boxes with gas-generating packets (Biomérieux, Lyon, France). MIC were determined using the E®-test strips (Biomérieux), as described by the manufacturer. In short, inoculates were adjusted to the turbidity of a 0.5 MacFarland standard and plated on Mueller-Hinton agar supplemented with 5% sheep blood (BioRad, Hercules, CA). Metric antimicrobial strips were applied, two per plate, in opposite direction. Tested antimicrobials were ampicillin (Amp), tetracycline (Tet), nalidixic acid (Nal), erythromycin (Ery), gentamycin (Gen) (all 0.016–256 μg/mL), and ciprofloxacin (Cip; 0.002–32 μg/mL). Plates were incubated for 48 h at 37°C. *C. jejuni* American Type Culture Collection (ATCC) 33560 was used as control strain, and was included in each batch during E-test.

The MIC was defined as the lowest concentration of an antimicrobial agent that completely inhibited visible growth

Table 2. Primers

Primer	*Sequence*
23SrRNA_F[a]	TTAGCTAATGTTGCCCGTACCG
23SrRNA_R[a]	AGCCAACCTTTGTAAGCCTCCG
Ery2075_R[a]	TAGTAAAGGTCCACGGGGTCGC
Ery2074_R[a]	AGTAAAGGTCCACGGGGTCTGG
Ery2074-2075_WT_R[a]	TAGTAAAGGTCCACGGGGTCTTT
Campjej_gyrA1_F[b]	TTTTTAGCAAAGATTCTGAT
Campjej_gyrA5_R[b]	CAAAGCATCATAAACTGCAA
Campjej_gyr_WT_F[b]	CAAAGCATCATAAACTGCAG
Campcol_gyr_F[b]	CACTTCCTGACGCTAGAGAT
Campcol_gyr_R[b]	TAAGGCATCGTAAACAGCCA
Campcol_gyr_WT_R[b]	TAAGGCATCGTAAACAGCCG
MD16S1[c]	ATCTAATGGCTTAACCATTAAAC
MD16S2[c]	GGACGGTAACTAGTTTAGTATT
MDmapA1[c]	CTATTTTATTTTTGAGTGCTTGTG
MDmapA2[c]	GCTTTATTTGCCATTTGTTTTATTA
Col3[c]	AATTGAAAATTGCTCCAACTATG
MDcol2[c]	TGATTTTATTATTTGTAGCAGCG

[a]Alonso *et al.* (2005).
[b]Zirnstein *et al.* (1999, 2000).
[c]Denis *et al.* (1999, 2001).

and was read at the point where the elliptical zone of inhibition intersected the MIC scale on the strip. Different measures were used to study resistance. EUCAST epidemiological cut-off values were used as the most sensitive measure of resistance development; different cut-off values are defined for *C. coli* (Amp >8, Tet >2, Nal >32, Ery >16, Gen >2, Cip >1) and *C. jejuni* (Amp >8, Tet >2, Nal >16, Ery >4, Gen >1, Cip >1). Multi-resistance was defined as simultaneous resistance to at least four antimicrobials tested.

Mutation analysis

The *gyr*A C257T mutation and 23S rRNA A2074C and A2075G mutations were examined by Mismatch Amplification Mutation Assay (MAMA) as described by Zirnstein *et al.* (1999, 2000) and Alonso *et al.* (2005), respectively. Primers are listed in Table 2. Resistant strains showing no mutations by MAMA were verified by sequence analysis, using Big Dye Terminator mix (Applied Biosystems, Carlsbad, CA) and analyzed on an ABI 3130 genetic analyzer.

Statistical analysis

Significance of differences between proportions of resistant isolates were evaluated using a χ^2-test with Yates continuity correction for large sampling groups ($N > 20$), whereas the two-tailed Fisher exact test was used for small sampling groups ($N < 20$). The 5% significance level was used (p-value < 0.05) for all statistical analyses.

Results and Discussion

A 6-year surveillance of randomly sampled Belgian pork and poultry meat products at the level of slaughterhouse, processing, and retail resulted in 1724 *Campylobacter* isolates. This isolate collection encompassed *C. jejuni* ($n = 1096$) and *C. coli* ($n = 377$) from poultry carcasses and meat, and *C. coli* ($n = 251$) from pork carcasses. Trends per species, per food matrix, per antimicrobial, and per year were analyzed. It should be mentioned that it is difficult to compare results with data from other countries due to the unavailability of comparable data. With the exception of Denmark, other European countries only started reporting *Campylobacter* antimicrobial resistance data from meat since 2006–2007 (EFSA, 2010). Furthermore there is a 2-year time lag of the European data analysis.

Resistance trends per antimicrobial

Yearly trends in resistance per antibiotic are shown in Figure 1. Ampicillin resistance fluctuated around the same resistance levels for all three species-matrix combinations, with an average resistance proportion of 13.1% and 30.8% for *C. coli* from pork and poultry meat, respectively, and 37.4% for *C. jejuni* from poultry meat. Tetracycline resistance showed yearly fluctuations around the same level for all three species-matrix combinations, with a significantly higher average resistance (p-value < 0.05) for *C. coli* (76.9% and 84.1% for isolates from poultry and pork meat, respectively) compared to *C. jejuni* (average resistance proportion of 40.8%).

Gentamicin resistance showed a significant increase between 2007 and 2009 for all three species-matrix combinations, from 0% in 2007, to over 20% in 2009. The most recent surveillance data from the European summary report does not show this high gentamicin resistance among *C. jejuni* from broiler meat (EFSA, 2010). None of the member states reported any gentamicin resistant isolates from broiler meat (EFSA, 2010). The observed increase of resistance against aminoglycoside antibiotics (like gentamicin) in all species-matrix combinations highlights the potential risk on further spread of the resistance among human pathogens. Since the introduction of the aminoglycoside apramycin for agricultural use (early 1980s), resistance to apramycin has emerged among *Escherichia coli* isolates found in cattle and pigs in France and the United Kingdom (Chaslus-Dancla and Lafont, 1985; Wray *et al.*, 1986). The aminoglycoside 3-*N*-acetyltransferase type IV resistance gene (AAC(3)IV) and 1-*N*-acetyltransferase (AAC(1)) confer resistance to several aminoglycoside antimicrobials (apramycin, tobramycin, gentamicin, kanamycin, and neomycin) and were first observed in animal isolates after the introduction of apramycin usage (Hedges and Shannon, 1986). Although apramycin has never been used for the treatment of infections in humans, these resistance determinants were subsequently found in human clinical isolates of *E. coli, Salmonella enterica*, *Klebsiella pneumoniae,* and *Campylobacter* spp. (Johnson *et al.*, 1994; Gomez-Lus *et al.*, 1999). This indicates that the usage of apramycin in agriculture primarily selected for the emergence of the aminoglycoside resistance genes among food animals, which then spread horizontally to human pathogens, where gentamicin is used for treatment.

Trends in resistance to erythromycin of all three species-matrix combinations were stable or decreased from 2004 to 2007, but increased in 2008 and 2009 to a resistance proportion of near 20.0% for *C. coli* and 12.1% for *C. jejuni*. Looking at the (fluoro)quinolone resistance proportions, the yearly trends for both *Campylobacter* species and both matrices were similar for both ciprofloxacin and nalidixic acid. Resistance in *C. jejuni* from poultry meat and *C. coli* from pork fluctuated around 40% for the whole study period. *C. coli* from poultry meat showed a consistently higher resistance for both antimicrobials, fluctuating around 60% for 2004–2007, peaking in 2008 (81.4% Cip and 86.0% Nal), and slightly decreasing in 2009. Belgian *C. jejuni* fluoroquinolone resistance proportions were significantly higher (p-value < 0.05) than reported in poultry meat in Denmark (19% fluoroquinolone resistance), yet similar to poultry meat imported in Denmark (53% fluoroquinolone resistance), yet lower than observed in poultry meat from Austria and Latvia (65% and 100% fluoroquinolone resistance, respectively) (DANMAP, 2008; EFSA, 2010). The observed trends of resistance proportions for all antimicrobials, specifically the distinct peaks for 2008, could result from changes in therapeutic use of these antimicrobials in animal food production during 2008–2009. Unfortunately, this hypothesis cannot be corroborated since detailed surveillance data of antimicrobial use in Belgian agriculture are not yet available.

Although antimicrobial resistance was monitored as acquired reduced susceptibility (using EUCAST epidemiological cut-off values), which cannot be used to deduce clinical implications, these observed high resistance proportions could have important human health consequences as erythromycin and ciprofloxacin are the preferred treatment for diagnosed cases of campylobacteriosis (Belanger and Shryock, 2007). In addition, most diarrheic patients that seek medical attention—and are not diagnosed with campylobacteriosis—are empirically treated with a broad-spectrum antimicrobial (Belanger and Shryock, 2007), most frequently with fluoroquinolones (Engberg *et al.*, 2004), for which especially *C. coli* from poultry meat shows a high proportion of resistance.

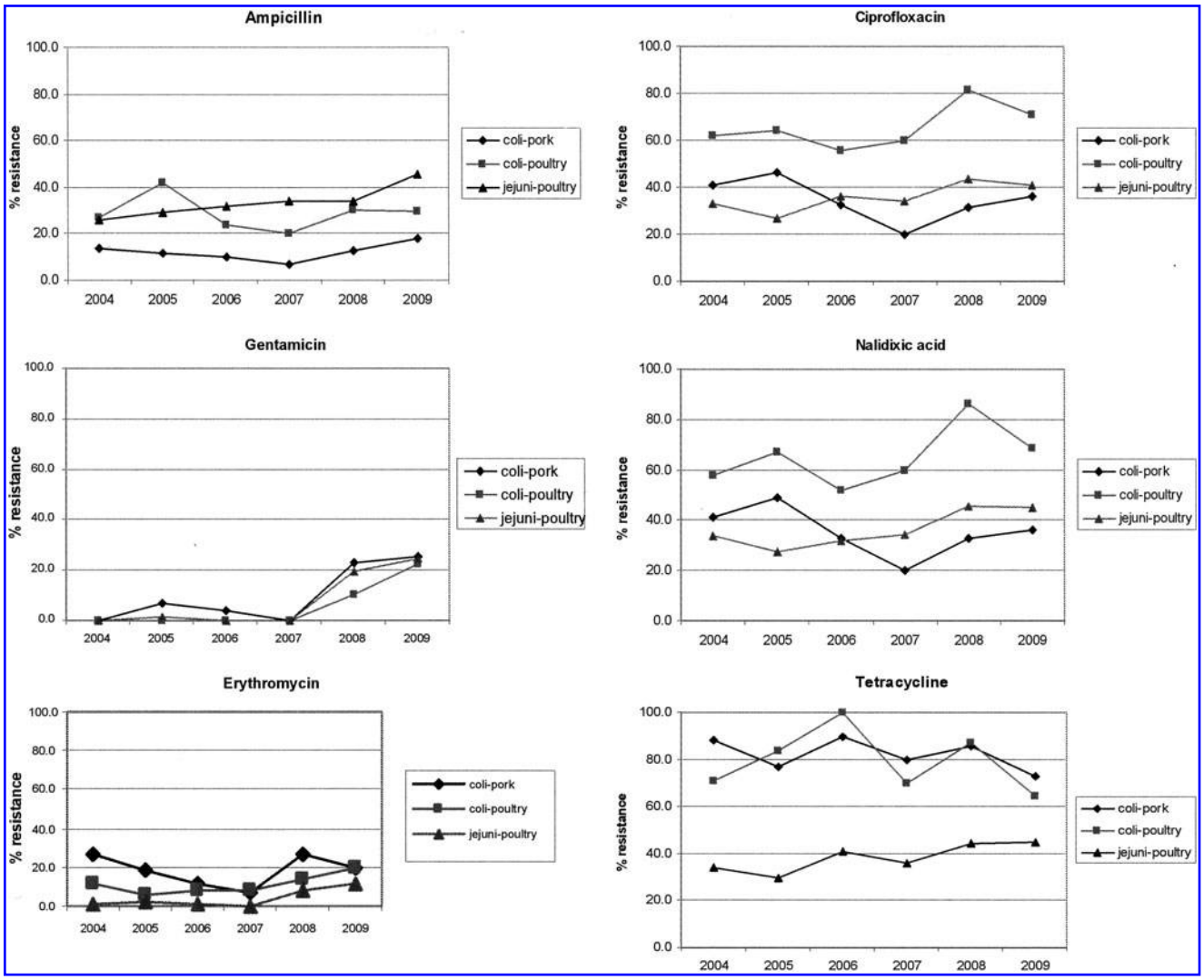

FIG. 1. Yearly trends of resistance to ampicillin, ciprofloxacin, nalidixic acid, tetracycline, gentamicin, and erythromycin for *Campylobacter coli* isolated from pork, and *C. coli* and *C. jejuni* from poultry. The epidemiological cutoff values as stated in the text were used.

Resistance trends per species and matrix

Average resistance per species and matrices were calculated for the whole study period based on MIC distribution (Table 3). In general, resistance proportions of *C. coli* and *C. jejuni* to the tested antimicrobials from poultry meat are in agreement with the average European results per year, which were based on the results of only a few member states (five for *C. jejuni* and three for *C. coli*) and to which Belgium contributed antimicrobial resistance results of 2006-2009. In Belgium during the study period 2004-2009 comparison of the two monitored meat matrices revealed significantly higher average resistance proportion (p-value < 0.05) to tetracycline (84.1% for pork and 76.9% for poultry) and erythromycin (17.9% for pork and 13.0% for poultry) for *C. coli* than *C. jejuni* (40.8% and 6.0% respectively). Significant differences in resistance proportion between matrices was not observed, except for ciprofloxacin and nalidixic acid, for which *C. coli* isolates from poultry showed higher resistance proportion (69.5% and 69.8% respectively) compared to *C. coli* from pork (both 35.5%). Furthermore, significantly higher resistance to ampicillin was observed in poultry isolates (30.8% for *C. coli* and 37.4% for *C. jejuni*), compared to pork isolates (13.1% for *C. coli*). Although detailed surveillance data on antimicrobial use in agriculture in Belgium are not available, some reports show trends which could explain the observed difference of fluoroquinolone resistance between pork and poultry food matrices. A study conducted by Timmerman *et al.* (2006) on the antimicrobial drug consumption at 821 Belgian pig herds revealed that nearly no fluoroquinolones are used in pig husbandry ($TI_{UDD} < 0.1$, with TI_{UDD} being the treatment incidence based on the Used Daily Dose or the number of pigs treated/1000 pigs at risk/day). The antimicrobial use in Belgian broiler production shows significant use of fluoroquinolones (enrofloxacin, $TI_{UDD} = 6.1$; flumequine, $TI_{UDD} = 1.39$) (Persoons and Dewulf, 2010). These data are consistent with results from monitoring programs on antimicrobial use of Denmark and The Netherlands (DANMAP, 2008; MARAN, 2007). Similarly, the use of β-lactam antimicrobials is higher in poultry- than in pig-husbandry (TI_{UDD} 37.9 and 28.9, respectively) (Persoons and Dewulf, 2010; Timmerman *et al.*, 2006).

TABLE 3. RESISTANCE PROPORTION FOR EACH MATRIX-SPECIES COMBINATION DURING THE 2004–2009 TEST PERIOD[a]

Resistance (%) of *Campylobacter coli* from pork meat

Antimicrobial agent	*%R*[b,c]	*2004*[d] (n=*22*)	*2005*[d] (n=*43*)	*2006*[d] (n=*49*)	*2007*[d] (n =*15*)	*2008*[d] (n=*70*)	*2009*[d] (n=*55*)
Ampicillin	13.1	20.0	11.6	10.2	6.7	12.9	16.7
Ciprofloxacin	35.5	40.0	48.8	32.7	20.0	31.4	35.2
Erytromycin	17.9	25.0	18.6	12.2	6.7	21.4	18.5
Gentamycin	13.9	0,0	7.0	4.1	0.0	22.9	25.9
Nalidixic acid	35.5	40.0	48.8	32.7	20.0	31.4	35.2
Tetracycline	84.1	100.0	81.4	89.8	80.0	85.7	74.1

Resistance (%) of *Campylobacter coli* from poultry meat

Antimicrobial agent	*%R*[b,c]	*2004*[d] (n=*34*)	*2005*[d] (n=*67*)	*2006*[d] (n=*25*)	*2007*[d] (n=*50*)	*2008*[d] (n=*86*)	*2009*[d] (n=*115*)
Ampicillin	30.8	36.7	41.8	24.1	20.0	30.2	29.6
Ciprofloxacin	69.5	63.3	64.2	62.1	60.0	81.4	71.3
Erytromycin	13.0	13.3	6.0	6.9	8.0	14.0	20.0
Gentamycin	9.3	0.0	0.0	0.0	0.0	10.5	22.6
Nalidixic acid	69.8	60.0	67.2	58.6	60.0	86.0	68.7
Tetracycline	76.9	73.3	83.6	96.6	70.0	87.2	64.3

Resistance (%) of *Campylobacter jejuni* from poultry meat

Antimicrobial agent	*%R*[b,c]	*2004*[d] (n=*163*)	*2005*[d] (n=*139*)	*2006*[d] (n=*66*)	*2007*[d] (n=*111*)	*2008*[d] (n=*313*)	*2009*[d] (n=*292*)
Ampicillin	37.4	37.0	29.5	32.9	34.2	34.2	47.4
Ciprofloxacin	38.0	33.3	26.6	34.1	34.2	43.5	42.6
Erytromycin	6.0	1.2	2.2	1.2	0.0	8.0	12.1
Gentamycin	12.9	1.9	1.4	0.0	0.0	19.8	25.6
Nalidixic acid	39.5	34.0	27.3	30.5	34.2	45.7	46.4
Tetracycline	40.8	35.8	29.5	46.3	36.0	44.4	45.3

[a]Results from 2004–2009 were summated.
[b]European Committee on Antimicrobial Susceptibility Testing epidemiological cut-off values were used to assign resistance.
[c]Average %R.
[d]Resistance (%) per year.

Multiresistance

Multiresistance (defined as resistance to four or more of the monitored antimicrobials) of the tested *Campylobacter* isolates is shown in Figure 2. Multiresistance of *C. coli* from poultry was on average high (27.6%), but showed strong fluctuations during the monitoring period. *C. coli* from pork and *C. jejuni* from poultry showed significantly less multiresistance, with less variation (11.2% and 15.8%, respectively). For both *C. coli* and *C. jejuni* from poultry, isolates resistant to all tested

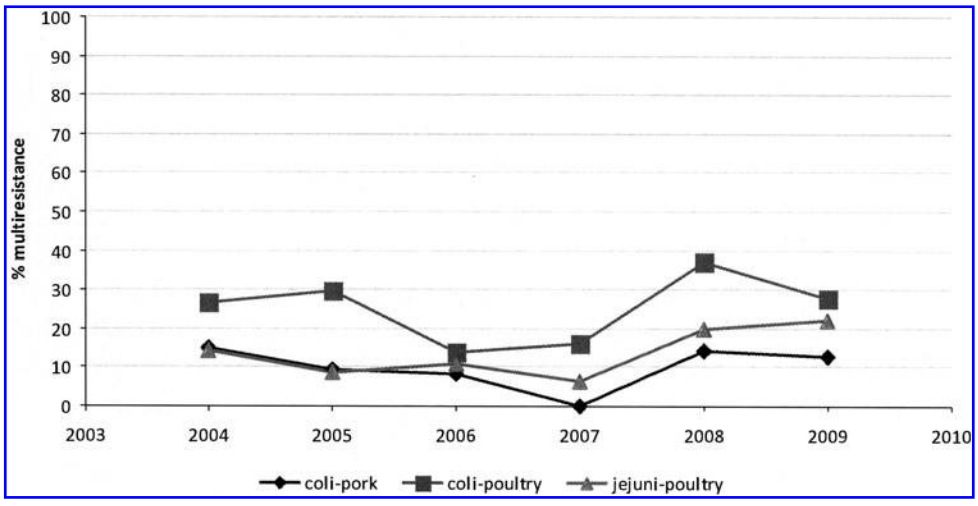

FIG. 2. Yearly trends in multiresistance per species-matrix combination. Multiresistance is defined as resistance to four or more of the tested antibiotics.

antimicrobials were observed (0.8% and 1%, respectively), whereas none were found in pork. Pan-susceptibility was significantly lower in *C. coli* (8% and 5% in pork and poultry, respectively), compared to *C. jejuni* (29% in poultry).

Resistance to (fluoro)quinolones (like ciprofloxacin and nalidixic acid) in *Campylobacter* spp. is mainly caused by mutations in the DNA gyrase A gene (Payot *et al.*, 2006). Unlike in other Gram-negative bacteria, where high level resistance is acquired by stepwise accumulation of point mutations, a single mutation in the quinolone-resistance-determining region (QRDR, C257T mutation) suffices to confer resistance (Luo *et al.*, 2003). For 97.5% of isolates, out of a selection of 89 ciprofloxacin-resistant strains, the C257T mutation was confirmed by mismatch amplification mutation assay–PCR (MAMA-PCR). Only two ciprofloxacin-resistant strains showed the wild-type *gyr*A gene with a cytosine at position 257.

The most common mechanism for macrolide resistance (like erythromycin) is target modification through point mutations in the 23S rRNA genes (A2074C and/or A2075G), of which there are three copies. MAMA-PCR showed the presence of the A2075G mutation in approximately 60% of the selected erythromycin-resistant strains, while the A2074C mutation was absent in all isolates. The sequence of the 23S rRNA gene of the remaining 40% of erythromycin-resistant strains was determined. All strains showed the wild-type adenine at position 2074 and 2075, indicating that another mechanism is responsible for the observed erythromycin resistance. Both laboratory studies and surveillance programs showed that fluoroquinolone resistance-conferring mutations in the *gyr*A gene of *Campylobacter* spp. result in a fitness advantage (Luangtongkum *et al.*, 2009). For instance, Luo *et al.* (2005) observed that quinolone-resistant *C. jejuni* isolates repeatedly outcompeted susceptible strains in the gut of a chicken animal model, demonstrating the positive effect of resistance on the fitness of this strain. On the other hand, resistance to macrolides are reported to cause a fitness burden, resulting in macrolide-resistant strains that will be outcompeted by natural strains once antibiotic pressure disappears (Caldwell *et al.*, 2008). Regardless of the potential biological relationship between virulence and antimicrobial resistance in *Campylobacter* spp., the clinical evidence suggests a bigger health burden due to infections caused by resistant strains (Aarestrup *et al.*, 2008).

Furthermore, *Campylobacter* spp. develop resistance to β-lactam antimicrobials by expression of β-lactamases (chromosomal OXA-type) and to tetracyclines through expression of the ribosome conformational modifying protein Tet(O) (Avrain *et al.*, 2004). However, apart from these individual resistance mechanisms, the expression of the energy-dependent multidrug efflux system CmeABC is known to be widespread among *Campylobacter* spp. (Olah *et al.*, 2006). Studies have shown that CmeABC expression confers resistance to fluoroquinolones, β-lactams, erythromycin, and tetracycline (with an increase of MIC by 8-, 32-, 4-, and 8-fold, respectively) (Lin *et al.*, 2002). The effect of CmeABC on aminoglycoside resistance (like gentamicin) is less apparent (Lin *et al.*, 2002). The most common antimicrobial multiresistance profiles found in our monitoring program were Amp-Cip-Nal-Tet and Cip-Nal-Ery-Tet, which are in agreement with the reported effect of the CmeABC multidrug efflux system.

Conclusion

Our study demonstrated an overall high antimicrobial resistance in *Campylobacter* isolates from meat. A remarkable increase of gentamicin resistance has been observed since 2007, which has thus far been unreported in poultry meat. Significantly more fluoroquinolone resistance is observed in meat from poultry compared to meat from pig, which is in agreement with the more frequent use of these antimicrobials in poultry husbandry. The C257T mutation of the *gyr*A gene was confirmed for nearly all fluoroquinolone-resistant isolates, whereas 40% of the erythromycin-resistant isolates in both *C. jejuni* and *C. coli* have the wild-type 23S rRNA gene. The high and increasing resistance proportions observed in this study, especially in poultry, combined with the high prevalence of *Campylobacter* in poultry carcasses at retail (25%, Table 1) are worrisome.

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Disclosure Statement

No competing financial interests exist.

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